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Electrostatic detection of unlabelled single- and double-stranded DNA using capacitive field-effect devices functionalized with a positively charged polyelectrolyte layer

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Abstract

Capacitive field-effect electrolyte-insulator-semiconductor sensors consisting of an Al-p-Si-SiO_2 structure have been used for the electrical detection of unlabelled single- and double-stranded DNA (dsDNA) molecules by their intrinsic charge. A simple functionalization protocol based on the layer-by-layer (LbL) technique was used to prepare a weak polyelectrolyte/probe-DNA bilayer, followed by the hybridization with complementary target DNA molecules. Due to the flat orientation of the LbL-adsorbed DNA molecules, a high sensor signal has been achieved. In addition, direct label-free detection of in-solution hybridized dsDNA molecules has been studied.

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Keywords: label-free detection; DNA biosensor; field-effect sensor; polyelectrolyte

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1. Introduction

Semiconductor field-effect devices (FEDs) based on an electrolyte-insulator-semiconducor (EIS) system are known as charge-sensitive devices [1-4] capable for the detection of charged molecules, like proteins or DNA (deoxyribonucleic acid), by their intrinsic molecular charge [5-10]. The major disadvantage of electrostatic DNA detection by the FEDs is, however, the screening of the negative charge of DNA molecules by counter ions in the solution [11,12].

In this work, a capacitive field-effect EIS sensor functionalized with a positively-charged weak polyelectrolyte layer of poly(allylamine hydrochloride) (PAH) was utilized for the electrical detection of unlabelled single- (ssDNA) and double-stranded DNA (dsDNA) by their intrinsic molecular charge. To achieve a preferentially flat orientation of DNA strands and thus, to reduce the distance between the DNA charge and EIS surface, the negatively charged DNA molecules were electrostatically adsorbed onto the positively charged PAH layer using a simple LbL technique. In this way, more DNA charge can be positioned within the Debye length, yielding a higher sensor signal.

2. Experimental

2.1. EIS sensor fabrication

The used sensor chips were fabricated from a p-Si wafer, beginning with a thermally dry oxidation process at 1000 °C for 30 minutes to grow a 30 nm thick SiO_2 gate oxide layer. After that, the rear-side oxide was removed via an etching step with HF followed by an Al layer deposition process to create an ohmic contact to the p-Si. Finally, the wafer was separated by cutting into single 10 mm × 10 mm chips. The chips were cleaned in an ultrasonic bath, consecutively filled with aceton, isopropanol, ethanol and deionized (DI) water for 3 minutes, respectively.

2.2. Surface functionalization and DNA adsorption via LbL technique

The cleaned EIS sensors were pretreated by a surface activation process with piranha solution: H_2SO_4 (98%) and H_2O_2 (35%) were pipetted directly onto the SiO_2 surface in a ratio of 1:3 for 10 minutes. After a thorough washing step with DI water, the surface was functionalized with a positively charged weak polyelectrolyte layer of PAH by pipetting 100 µL of PAH solution (50 µM in 100 mM NaCl, pH 5.4) to the SiO_2 layer for 10 minutes at room temperature (RT) followed by rinsing with DI water.

Two kinds of DNA solutions were used for the adsorption process: The first solution contains only probe ssDNA (40 mer, 5 µM in DI water), while the second solution contains a mixture of probe ssDNA (40 mer, 5 µM in DI water) and complementary target cDNA (20 mer, 1x PBS, 0.5 M NaCl). Both solutions were heated up to 95 °C for 5 minutes in a water bath, then directly cooled down to 60 °C for 0.5 minutes in a second water bath. Then, they were removed from the water bath, cooled down to RT and pipetted (60 µL) on the PAH-modified sensor surface for 15 min. After DNA adsorption, the chip surface was rinsed with measurement solution (0.33 mM PBS (equivalent to 5 mM ionic strength), adjusted to pH 7 with NaOH). Figure 1 depicts the layer-by-layer adsorption process of the polyelectrolyte/DNA bilayer.

2.3. Electrochemical characterization

The sensor chips were characterized before and after each modification step by means of capacitance-voltage and constant-capacitance (ConCap) mode [11,12] measurements using a Zennium electrochemical workstation (Zahner Elektrik, Germany) in a two-electrode arrangement. Figure 2 shows a cross-sectional schematic of the modified EIS structure and measurement setup used for the electrochemical characterization.

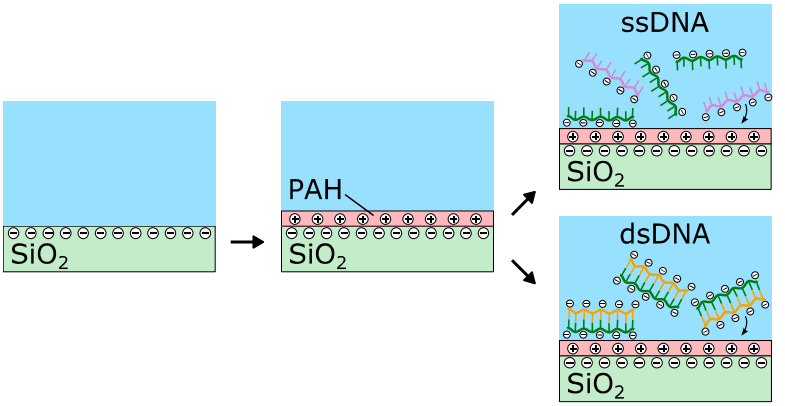

Fig. 1. Schematic of the LbL adsorption process of ssDNA or dsDNA molecules, respectively, onto the EIS sensor modified with a positively charged PAH layer.

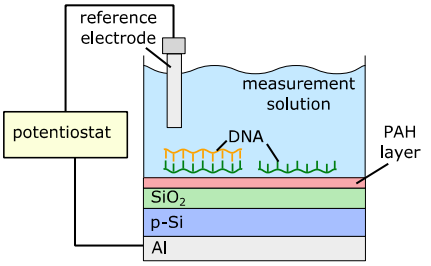

Fig. 2. Measurement setup for the characterization of EIS sensors (schematically).

3. Results and discussion

Figure 3 shows an example of label-free detection of ssDNA and dsDNA by means of EIS sensors modified with a PAH layer. In this experiment, the sensor signal for two different EIS sensor chips has been recorded in a ConCap mode before and after the polyelectrolyte adsorption as well as after adsorption of ssDNA (a) and in-solution hybridized dsDNA (b) molecules. A surface potential change of 24 mV was recorded after ssDNA adsorption (Fig. 3a), while it was nearly doubled (57 mV) after dsDNA adsorption (Fig. 3b). The results of the electrochemical characterization were supported by fluorescence microscopy measurements as additional reference method.

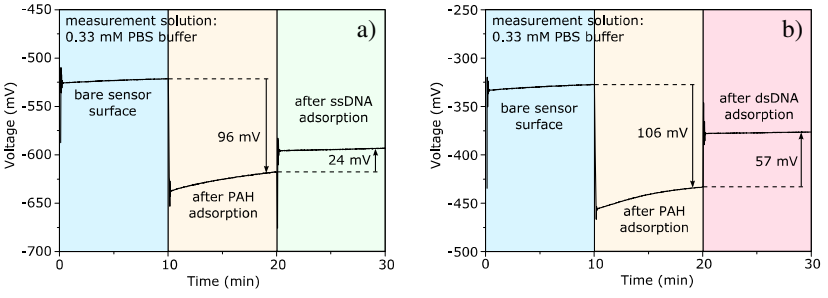

Fig. 3. ConCap response of two EIS sensors before and after polyelectrolyte adsorption as well as after adsorption of ssDNA (a) and in-solution hybridized dsDNA (b) molecules.

4. Conclusion

The adsorption of unlabelled negatively charged ssDNA and dsDNA onto EIS sensors modified with a positively charged polyelectrolyte layer was investigated. The obtained results demonstrate the potential of capacitive EIS sensors for future realization of simple detection tools capable for distinguishing between ssDNA and dsDNA adsorption.

Acknowledgements

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